LARGE-SCALE PEI-MEDIATED PLASMID TRANSFECTION

RELATED APPLICATIONS

[0001] This application asserts priority to Patent Cooperation Treaty application Serial No. PCT/US2017/025681 filed 3 Apr. 2017, which in turn asserts priority to United States provisional patent filing Ser. No. 62/322,651 filed 14 Apr. 2016, and the contents of each are here incorporated by reference.

GOVERNMENT INTEREST

[0002] None.

PRIOR DISCLOSURES

[0003] Not applicable.

BACKGROUND

[0004] Many vectors used in gene therapy, such as Lentiviral vectors and Adeno-associated Virus (AAV) are commonly produced by co-transfecting adherent HEK 293T cells with several different plasmid constructs (Follenzi and Naldini, 2002; Tiscornia et al., 2006; Chiorine et al. 1999). The most commonly used reagent in plasmid transfection is calcium phosphate (Tiscornia et al., 2006; Follenzi and Naldini, 2002; Reiser, 2000; Koldej et al., 2005; Naldini et al., 1996a; Sena-Esteves et al., 2004). Alternatively other reagents, like an activated-dendrimer based SUPERFECT™ (Coleman et al., 2003) or N,N-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) (Karolewski et al., 2003), have been used. Polyethylenimine ("PEI") mediated transfection has also gained interest (Kuroda et al., 2008, Segura et al 2007, Chahal et al. 2014).

[0005] Many applications have still relied on flask type two dimensional (2D) approaches such as Cell Factories. Production up-scaling in flasks is limited by the production space required and multiple units makes it impractical to handle and difficult to monitor/control culture conditions. Microcarriers have also been tried (Wu et al., 2002), dispersed in suspension but have not proven easy enough to handle to ensure homogenous growth. A critical limitation has been the expansion of a large cell mass on a static vessel, a process with limited scalability. This approach also needs labor-consuming operations for the separation and purification of the vector from the producer cells later in the process. (Dormond et al., 2009).

[0006] The use of packed-bed bioreactors have provided three dimensional (3D) controlled, perfusable systems with low shear stress for adherent (and suspension) cells (Meuwly et al., 2007). A novel fixed-bed bioreactor, the iCELLis® provides a recent development providing from 66 m^2 to 500 $m^2$of a polyethylene terephthalate (PET) matrix substrate for adherent cell growth (FIG. **1**). (N.B.: In our patent, we use the term "substrate" not in the enzymology sense of a compound which is changed by an enzyme, but in the cell culture sense of a material providing a solid surface to which cells can adhere and grow in adherent mode, for example a polymer matrix or other macrocarrier) The iCELLis® Nano has been used for a range of vector applications, such as for AAV (Lennaertz et al., 2013), retrovirus (Wang et al., 2015), Rabies, Hepatitis-A and Chikungunya vaccine production (Rajendran et al., 2014). Previously, we evaluated for the first time the fixed bed iCELLis® bioreactor for the manufacturing of Ad5 vectors in a HEK293 cell line (Lesch et al. 2015). The process development was started in an iCELLis® Nano and for the first time we achieved efficient scale up of the manufacturing into iCELLis® 500 large scale equipment. A surprising finding at the time was to use suspension techniques to expand the cell mass for adherent bioreactor where the cells attached onto the macrocarriers and continued the growth in an adherent more (Patent number GB14/17042.7 and PCT. US2015.46927). By using this approach iCELLis® 500 can provide up to 500 m2 of cell culture area in adherent mode to meet the good manufacturing practices (GMP) requirements for the manufacturing of commercial scale product.

[0007] Even though several suspension approaches are available for many viruses (Kamen et al., 2004, Ferreira et al., 2005, Cortin et al. 2004, Liu et al., 2009), the adherent HEK293 or HEK293T cell line is often crucial because the productivity of the specific vector in adherent mode can be much higher than in suspension. The use of FBS may not be a desired trend, but in some occasion, the addition of FBS was needed to increase the productivity and is thus essential. This phenomenon has seen previously with adenovirus (Iyer et al., 1999) and other virus types, especially with enveloped viruses. For example, the lipids were shown to be a key serum component during retroviral vector production to increase the yield and vector stability (Rodrigues et al. 2009). Understanding the cell metabolism and the deprivation of serum or replacing it with synthetic molecules are constantly increasing area of interest (Petiot et al., 2015). In addition, there are some cell lines that cannot be grown is suspension mode, so adherent systems are the only possibility.

[0008] The need for large scale adherent manufacturing is clear. The iCELLis® fixed-bed bioreactor with 3D PET matrix provides homogenous media control and an effective head-space gassing system. The system provides a Single Use System ("SUS") comprising a readily disposable cassette housing the PET adherent culture substrate, combined with medium perfusion capability and with automated control of stirring, temperature, pH and dissolved oxygen, which it can minimize batch-to-batch variation. We tested the iCELLis® fixed bed bioreactor and optimized it for adenovirus production in a small scale and then scaled up into a large scale 100 m^2 bioreactor (Lesch et al 2015). The iCELLis® 500 provides the process in a disposable manner with all probes and tubing delivered sterile and disposable. This is highly desirable for GMP manufacturing, as with disposable systems, there are no regulatory requirements to validate the product specific equipment cleaning or sterilization. The preparation of the equipment was fast and the risk of contamination was minimized with the closed system transfers. It was easy to set up and use.

[0009] Transient transfection method in a small scale is straightforward to perform, versatile and avoids the time-consuming development of stable cells lines. It also allows easy and rapid testing of various transgenes or pseudotypes (Sena-Esteves et al., 2004). Adherent large scale production with plasmid transfection has been achieved using 10 layer cell factories (Geraerts et al., 2005; Slepushkin et al., 2003). The scalability of any flask type approach, however, is limited. Also, the scalability of the transfection itself may become a challenge. We have recently figured out how to use an iCELLis™-type bioreactor for manufacturing of lentivirus and AAV using a calcium phosphate- or PEI-mediated